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Research Article

DETERMINATION OF ESSENTIAL OIL CONTENT AND ANTIOXIDANT CAPACITY OF RHIZOMA ET RADIX NOTOPTERYGII

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ABSTRACT: The present paper examined the essential oils content and antioxidant capacity of Rhizoma et Radix Notopterygii, and the essential oils were obtained by steam distillation. The antioxidant activities were investigated in two aspects: the capacity of removing superoxide anion radical and the clearance of hydroxyl radical, which were tested by 1,10-Phenanthroline Spectrophotometry and the visible light-mediated pro-oxidant of Vitamin B2. Results showed that the content of essential oils ranged from 0.5% to 4.6% (ml/100 g). All examined oils exhibited a free radical scavenging activity, the O_2^- clearance and . OH inhibition rate was between 0.85% - 7.08% and 2.73% - 3.68%, respectively. The results demonstrated that the combination of essential oil content and antioxidant capacity determination is effective in quality control of Rhizoma et Radix Notopterygii.

Key words: Rhizoma et Radix Notopterygii; Essential oil; Determination; Antioxidant

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INTRODUCTION

Radix et Rhizoma Notopterygii (in Chinese: Qianghuo), is the root of *Notopterygium incisum* Ting ex H. T. Chang or *Notopterygium franchetii* H. de Boiss (Chinese Pharmacopoeia, 2010). Rhizoma et Radix Notopterygii is specific to China, and mainly grow up in Qinghai, Gansu, Shanxi, Sichuan and Yunnan (Jin PP, 2011). It is a perennial herb that has been widely used in traditional Chinese medicine for the treatment of pain relieving, dispersing coldness and dampness. In addition, Radix et Rhizoma Notopterygii was used in some ethnics, such as Tibetan and Qiang minorities. Previous researches have studied much on chemical compositions of essential oil from Radix et Rhizoma Notopterygii. However, there is no report about the antioxidant capacity of aqueous extracts, and evaluated by 1, 10-Phenanthroline Spectrophotometry and the visible light-mediated pro-oxidant effects of Vitamin B_2. The biological activity of Notopterygii such as antimicrobial (Noriko Sato, 2002) and anti-inflammatory (Zhang MF, et al., 1996) have been investigated. But there is no research on the antioxidant activity of the essential oils of Notopterygium. With the development of society, we are surrounded by the kinds of pollution, which increased the damage of free radicals. The food, health products and medicines are used to reduce the risk by many people (Kris-Etherton PM, et al., 2002).

With the emerging of ·OH and $\cdot O_2^-$, which lead to cells' damage and degradation. Free radicals oxidative stress damage theory is a hotspot in recent years, which are very closely related to many neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington disease and so on (Jing P, 2008). These diseases are global challenges, and need to overcome as soon as possible. Therefore, it is very crucial for detecting the content of essential oil and its antioxidant activity to the global.

In our study, we examined the essential oils content and antioxidant capacity of Rhizoma et Radix Notopterygii for the first time. The result is representative and may potentially guide authorities to control the quality of Rhizoma et Radix Notopterygii and provide the basis for new neurodegenerative drugs development.

MATERIALS AND METHODS

Chemical reagent and apparatus

1,10-Phenanthroline monohydrate, $FeSO4 \cdot 7H_2O$, H_2O_2 (30%), KH_2PO_4, Nitro blue tetrozolium (NBT), NaOH (Sinopharm Chemical Reagent Co., Ltd), Volatile oil, UV2550 spectrophotometer (Shimadzu), heated water bath (Beijing Jingkehuarui Co., Ltd), vortex (Haimen Kylin-Bell apparatus Co., Ltd), Biotek Synergy 2 (Gene Company Limited). All chemical reagents are analytical grade.

Plant material

The samples were selected on the basis of different provinces and three counties were chosen in each province, plus three commercial use according to morphological differences (Table 1). The Rhizoma et Radix Notopterygium were collected in November, 2013 from 12 cities from Sichuan, Gansu, Qinghai province. Specimen were taxonomically identified by professor Linfang Huang and vouchers were deposited at the Herarium of Institute of Medicinal Plant Development (IMPLAD). The samples were dried in the local area after harvesting.

Isolation of the essential oil

According to the reported literature (Zhong G, 2008), the essential oils were obtained by hydro distillation. 90 g (Through No. 2 sieve) dried and ground powder of Rhizoma et Radix of Notopterygii together with 500 ml water were submitted to hydro distillation to a 1 L round-bottomed flask, 4 glass beads, and immersed for an hour, heated gently till to boiling in a heating thermostat set, maintained about 6 hours up to the oil no longer increased. At the end of each distillation the oils were collected, dried over anhydrous Na_2SO_4 and after filtration, measured and kept at a temperature of +4°C for further analysis. The respective colors of volatile oils at first were transparent, and turned to light green gradually. On the day of assay, the extracts were vortexed and cooled to room temperature. The yield of the different producing areas was counted by Table 1. Full details are provided in Table 1.

Table 1: The yield of essential oil of Notopterygii.

Source	Latin names	Account of essential oil % (ml/100 g)	Description	Collection date	Abbreviation
Banma, Qinghai	*N. incisum*	0.9	wild	Oct, 2013	BM
Gande, Qinghai	*N. incisum*	0.5	wild	Oct, 2013	GD
Dari, Qinghai	*N. incisum*	0.7	wild	Oct, 2013	DR
Aba, Sichuan	*N. incisum*	4.6	wild	Oct, 2013	AB
Rangtang, Sichuan	*N. incisum*	2.4	wild	Oct, 2013	RT
Dege, Sichuan	*N. incisum*	2	wild	Oct, 2013	DG
Kangding, Sichuan	*N. incisum*	3.4	wild	Oct, 2013	KD
Xining[1], Qinghai	*N. franchetii*	2.7	wild	Oct, 2013	TQ
Xining[2], Qinghai	*N. franchetii*	2.7	wild	Oct, 2013	DQ
Minxian, Gansu	*N. franchetii*	1.7	cultured	Oct, 2013	MX
Weiyuan, Gansu	*N. franchetii*	1.9	cultured	Oct, 2013	WY
Lintao, Gansu	*N. franchetii*	1.1	cultured	Oct, 2013	LT

The discrimination of morphological identification (Guo XF, et al., 2010): Kangding was silkworm Notopterygium, Xining[1] was striped Notopterygium; Xining[2] was irregular-nodal Notopterygium

ANTIOXIDANT ACTIVITY

Riboflavine photosensitization

Use the buffer to prepare the radical system (Beauchamp C, et al., 1971; Beydemir S, et al., 2004): composition of 3.3×10^{-6} mol/L vitamin C, 0.01 mol/L Methionine, 4.6×10^{-5} mol/L NBT prepare when needed, protected from light. Mixed 3 ml of radical system and 0.1 ml essential oil from different producing areas together, 4000 lx light for 30 minutes at room temperature, with distillation water as reference solution, to detect the largest absorbance wavelength at 560 nm, A. Use the water to replace the essential oil, A_0, to calculate the clearance of radical according to the formula.

$$O_2^{\bullet} - \text{Clearance}\% = \frac{A_0 - A}{A_0} \times 100$$

1,10-Phenanthroline Spectrophotometry

The antioxidant activity was determined by 1, 10-Phenanthroline Spectrophotometry assay, using hydroxyl radical ·OH, according to the procedure described by Wang Duoning (Wang DN, et al., 2006) with some modification. The assay was performed by UV2550 and Biotek Synergy 2 simultaneously. Take 0.5 ml of 1,10-Phenanthroline (5 mmol/L) to 0.75 mol/L phosphate (pH 7.4) 2.4 ml, mixed on the vortex, pulsing 7.5 mmol/L Ferrous sulfate solution 0.5 ml, votexed after each ones, then add 0.1ml essential oil to test tube, add 3% H_2O_2 0.5 ml, finally. Keep the reaction solution in 310 K for half an hour, then measured at 510 nm. The experiment was divided into three groups: A_0 (no essential oil and no H_2O_2), A_1 (no essential oil but with H_2O_2), M (essential oil and H_2O_2).

To calculate the clearance of ·OH, i.e., I%

$$I\% = [(M - A1) / (A_0 - A_1)] \times 100$$

The antioxidant activity (.OH clearance) was performed on different instruments. The reaction solution was full-length-scanned by UV2550 spectrophotometer system. The max absorbance wavelength was 510nm in this situation. While in Biotek Synergy 2, combined with the reported literature (Guo XF, et al., 2010; Zhong F, et al., 2004; Liu X, et al., 2009), two wave-length 510 nm and 536 nm was chosen to detect the absorbance of the antioxidant of the reaction solution. The analysis was carried out using 96-well microplates, 100 µl were added. All the essential oil samples were analyzed at three times. All the data collected for each assay are the averages of three determinations of three independent experiments.

RESULTS AND DISCUSSION

The total essential oil's antioxidant activity of the different origins of the Rhizoma et Radix Notopterygii were shown in Table 1. The mean oil yields of Notopterygium. Incisum and *N. franchetii* were 0.5% ~ 4.6% and 1.1%~2.7% (ml/100 g), respectively (Table 1). Samples from Qinghai provinces were all lower than the required criteria of the Chinese Pharmacopoeia (2015 edition), 1.4% (ml/100 g), and so did the specimen from Lintao, Gansu province. Three commercial specifications were all fit the criteria of the Chinese Pharmacopoeia (2015 edition) ranging 2.7% from 3.4%, the striped and irregular-nodal Notopterygium were nearly the same content of essential oils. According to the Table 1, ABa has the most yields among the 12 batches of the samples.

Figure 1 showed the X axis, the origins of samples, the Y axis described the removing of superoxide anion capacity produced by the visible light-mediated pro-oxidant effects of Vitamin B_2, Riboflavine. The 12 batches of antioxidant capacity range 0.85% from 7.08% per gram. GD showed the lowest superoxide anion clearance, DG explained the highest capacity among 12 batches, while KD, AB and RT had the similar antioxidant capacity, samples from Qinghai province described the lower superoxide anion removing capacity compared to other two provinces.

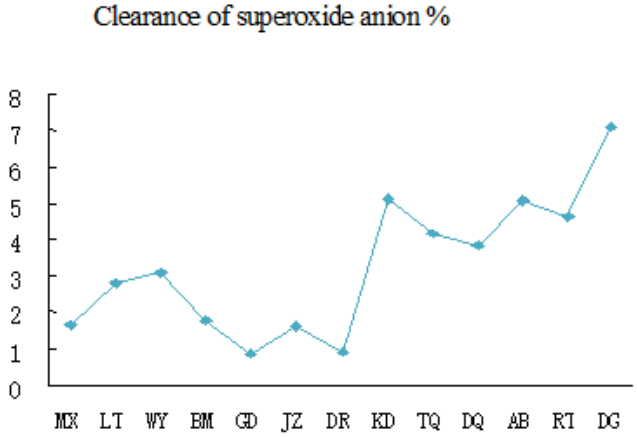

Figure 1: The clearance of superoxide anion.

Figure 2 described the same specimen detected by two instrument, left column was UV2550 detection value, and the right column was obtained by Biotek Synergy 2, the full length scan of 400 nm-800 nm by UV2550 showed that 510 nm have the largest absorption, so both of them were compared the 510 nm absorption values. The coefficient of variation between the triplet values of UV2550 was 1.09% - 35.64%, as a consequence of the low reproducible of the free hydroxyl radical clearance, and according to the reported literature (Xu D, et al., 2010), Biotek Synergy 2 was introduced to this experiment, e.g., Figure 2 showed that DQ has the most antioxidant capacity among the 12 batches by Synergy 2 and higher than UV2550 in the majority samples.

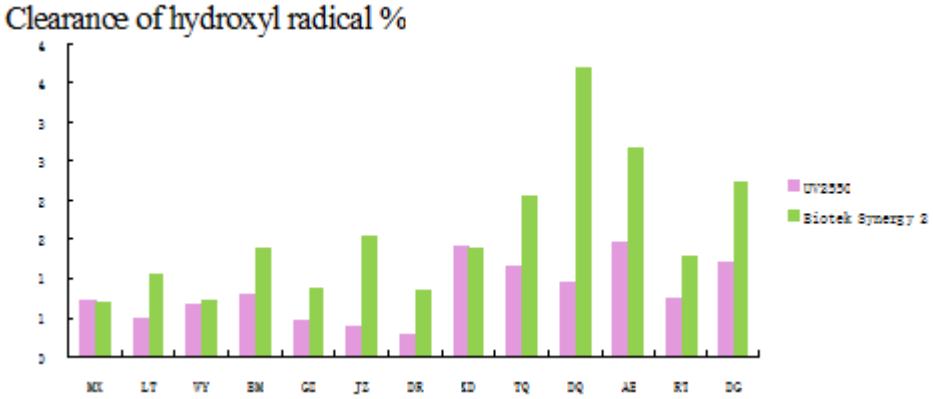

Figure 2: Different instrument under the 510 nm absorption.

Figure 3 described the two absorbance wavelength by Biotek Synergy 2, it clearly showed that 1,10-Phenanthroline Spectrophotometry had more absorbance under 536 nm than 510 nm. Both of the two wavelengths showed the same tendency of the absorbance, but under 536 nm detection had higher value than 510 nm. It indicated that 536 nm was more suitable than 510 nm in the hydroxyl radical clearance experiment. Compared Figure 2 with Figure 3, Synergy 2 was time-saving, convenient and with less amount of test samples, but the reuse of the 96 well microplate would minimize the quantitative of samples, Biotek Synergy 2 could be simultaneously detected at dual wavelength. According to Figure 2 and Figure 3, 536nm have the more absorption in the 1,10-Phenanthroline Spectrophotometry.

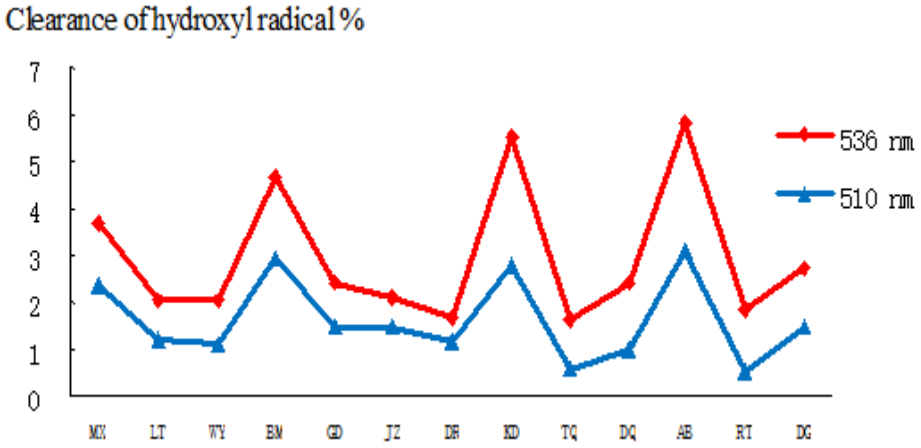

Figure 3: Comparison of two different wavelengths.

The capacity of clearing superoxide anion from the Figure 1 and Figure 3 of the three commercial specification, KD was in accord with in the removing of superoxide anion and hydroxyl radicals. During the clearance of hydroxyl radicals, cultured *N. franchetii* were superior to wild *N. franchetii*, while in the removing of superoxide anions, cultured N. franchetii were inferior to wild *N. ranchetii*. Among the three producing areas, Sichuan province has the most antioxidant activities. It was in accordance with traditional conception that Sichuan‘s Notopterygium is relatively good quality.

CONCLUSION

All examined oils in Rhizoma et Radix Notopterygii exhibited a free radical scavenging activity, ranging of 0.85 %- 7.08% of $O_2^{\bullet}$-clearance and 2.73%-3.68%. .OH inhibition.

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